

CHRONIC TOXICITY SUMMARY

4,4'-METHYLENE DIANILINE

(MDA; 4,4'-diaminodiphenylmethane; 4,4'-diphenylmethanedianiline; DAPM; dianilinmethane)

CAS Registry Number: 101-77-9

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	20 µg/m³ (2 ppb)
<i>Critical effect(s)</i>	Ocular toxicity to the retinas of guinea pigs
<i>Hazard index target(s)</i>	Eyes; alimentary system (hepatotoxicity)

II. Chemical Property Summary (HSDB, 1995; CRC, 1994)

<i>Description</i>	Colorless to pale yellow flakes; tan
<i>Molecular formula</i>	C ₁₃ H ₁₄ N ₂
<i>Molecular weight</i>	198.3 g/mol
<i>Boiling point</i>	398-399°C
<i>Melting point</i>	92.5°C
<i>Vapor pressure</i>	1 torr @ 197°C
<i>Solubility</i>	Soluble in alcohol, benzene, ether; 273 g/100 g acetone; 0.1 g/100 g water @ 25°C
<i>Conversion factor</i>	8.1 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

4,4'-Methylene dianiline (MDA) is synthesized by the reaction of aniline with formaldehyde. MDA's major uses are as a chemical intermediate in the synthesis of certain isocyanates and polyurethane polymers, as a corrosion inhibitor, in the preparation of azo dyes, as a rubber preservative, and in the curing of epoxy resins and neoprene (HSDB, 1995; ACGIH, 1992). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1133 pounds of MDA (and its dichloride) (CARB, 2000).

IV. Effects of Human Exposure

Several cases of human exposure to MDA have identified the compound as a hepatotoxicant which produces cholestatic jaundice (Kopelman *et al.*, 1966; McGill and Motto, 1974; Williams *et al.*, 1974; Bastian, 1984). Bastian (1984) described cases of acute hepatic illness in four workers exposed from laying floors using an epoxy resin base, which contained MDA as a curing agent. The workers were exposed via fumes and dusts in the air as well from hand

contact with powder and had worked with epoxy resins for periods ranging from one to 12 years. The level of exposure was not quantified. The workers initially reported to the hospital with symptoms of abdominal pain three days after the most recent exposure and all were discharged within four days. Two workers continued to show severe symptoms five days after the onset, with abdominal pain, jaundice, a tender liver, nausea, dyspnea, and muscular pain. Plasma bilirubin, alkaline phosphatase, and aspartate aminotransferase levels were elevated. Some symptoms did not subside until two months after the onset. One worker, after another exposure, experienced nausea, abdominal pain, and muscular pain. A second worker reported further symptoms of headache, tiredness, and decreased libido.

Williams *et al.* (1974) reported symptoms in 6 of approximately 300 workers exposed to MDA by surface coating concrete walls with epoxy resins. Exposure probably occurred by inhalation, ingestion, and skin contact as a result of mixing powder containing MDA. Symptoms of clinical hepatitis in the 6 workers appeared two days to two weeks after beginning work; five of the six had elevated bilirubin levels, and one liver biopsy showed bile stasis. All the workers recovered completely after an unspecified time.

McGill and Motto (1974) described hepatitis among 13 men who, over the course of 6 years, were occupationally exposed to MDA in the blending of epoxy resins used in the manufacture of insulating material. Among the 13 patients showing symptoms, all reported weakness, jaundice, and dark urine; 11 reported abdominal pain, nausea or vomiting, and anorexia; and over half reported fever, chills and/or headache. All the workers recovered within a 10 week period. After the first cases of hepatitis occurred, air sampling showed initial levels of MDA to be 0.1 ppm in the work area. After additional cases of hepatitis occurred, measures were taken to reduce worker exposure, and air levels were reduced to as low as 0.0064 ppm. The authors concluded that percutaneous absorption was the likely major route of exposure in light of the fact that cases occurred in spite of measures taken to reduce air levels and there was evidence that significant hand contact with the compound occurred during the workday. Since the symptoms appeared within one to 18 days after “working intensively” with the compound and exposure routes were not clearly established, quantitation of exposure levels was considered difficult.

The most well-known incident of MDA toxicity to humans resulted from ingestion of bread made with flour contaminated with MDA during transport (Kopelman *et al.*, 1966a). Eighty-four persons showed symptoms of abdominal pain and some degree of jaundice. All patients had elevated serum alkaline phosphatase and glutamic oxaloacetic transaminase levels. Seventeen had serum bilirubin levels over 5 mg/100 ml. Liver biopsy was performed on 8 persons and evaluated in a separate study (Kopelman *et al.*, 1966b). The primary finding was an unusual lesion described during the early course of the disease as portal zone cholangitis and later as centrilobular cholestasis with necrosis. The initial study reported that all but 2 patients had complete recovery within several weeks. However, a two year follow-up study of 14 individuals showed that 10 still had symptoms of some severity 7 to 23 months after initial onset including food intolerance, gastrointestinal disturbances, fatigue, and visual disturbances (Kopelman, 1968).

Human effects other than hepatotoxicity have been described including several cases of contact dermatitis and skin sensitization (LeVine, 1983; Van Joost *et al.*, 1987; de Pablo *et al.*, 1992;

Bruynzeel and van der Wegen-Keijser, 1993). A case report of a man exposed to MDA with potassium carbonate and γ -butyrolactone by accidental ingestion has been described (Roy *et al.*, 1985). In addition to hepatitis and abnormal liver function, which persisted over 18 months, the patient developed a progressively worsening retinopathy described as a “malfunction of the retinal pigment epithelium” accompanied by diminished visual acuity. The patient improved after approximately 3 months, but after examination at 18 months had not completely recovered.

Another report described the development of acute cardiomyopathy in addition to hepatitis in a worker exposed to a large quantity of MDA dust as the result of air filtration malfunction (Brooks *et al.*, 1979). The patient showed an abnormal ECG and an elevated cardiac LDH isoenzyme profile, which returned to normal within one month of onset.

V. Effects of Animal Exposure

The carcinogenicity of MDA was investigated in F344/N rats and B6C3F₁ mice (50/sex/dose group) administered in the drinking water at concentrations of 0, 150, and 300 ppm MDA (dihydrochloride) for 103 weeks (Lamb *et al.*, 1986). A 14-day range finding study was also conducted with 5 animal/sex/species/dose group, with exposure levels of 0, 200, 400, 800, 1600, and 3200 ppm MDA. A 13-week subchronic study was conducted with 10 animals/sex/species/dose group and exposure levels of 0, 25 (mice), 50, 100, 200, 400, and 800 (rats) ppm MDA. Using body weight and drinking water values from the study, low and high daily doses in the chronic study were calculated to be 9 and 16 mg/kg-day for male rats, 10 and 19 for female rats, 25 and 57 for male mice, and 19 and 43 for female mice. In the chronic study, survival was reduced among male mice treated with 300 ppm MDA. Final mean body weights were reduced in the 300 ppm dose group of female rats (-9%), male mice (-13%), and female mice (-16%). Among rats, non-cancer effects included follicular cysts and follicular-cell hyperplasia of the thyroid (significantly increased incidence in high-dose females; $p < 0.05$ by Fisher's exact test). In the liver, the incidence of fatty and focal cellular change was elevated in low-dose male and female rats and also in high dose male rats. Incidence of unspecified dilatation of the liver was also elevated in high-dose male rats. Increased incidence of kidney mineralization was found in male rats treated with 300 ppm MDA. Among mice, incidence of liver degeneration was elevated in males in both treatment groups and females in the high-dose group ($p < 0.01$ by Fisher's exact test). Incidence of kidney nephropathy was increased in male and female mice in both treatment groups and mineralization of the renal papilla was increased in both sexes in the high-dose group ($p < 0.01$). From the 13-week study, the authors noted thyroid and bile duct effects in rats at 800 ppm MDA in water and in mice at 400 ppm MDA in water.

Albino and pigmented guinea pigs were exposed to aerosols of methylene dianiline in polyethylene glycol 200 (PEG) in nose-only exposure chambers (Leong *et al.*, 1987). Animals (8 of each strain) were exposed to a time-weighted average aerosol concentration of 0.44 g MDA/m³ in air for 4 hours/day, 5 days/week for 2 weeks. Eight control animals were neither exposed to aerosol nor placed in the exposure chamber. Two weeks after the exposure period, animals were evaluated for dermal sensitization and irritation by challenge with 0.05 ml of 0, 2, 20, and 200 mg MDA/ml in PEG for up to 24 hours. No evidence of dermal irritation or

sensitivity was found. Subsequently, the animals were also examined for pulmonary sensitization by challenge with aerosols containing 0.01 and 0.05 ml of 200 mg MDA/ml PEG. Lung insufflation pressures were measured as an indication of changes in lung distensibility. No evidence of pulmonary sensitization was found. After the pulmonary challenge, the animals were examined histopathologically, with emphasis on eye, lung, liver, and kidney toxicity. Ocular toxicity ranging from mild to more severe was observed in all MDA-treated animals, but in none of the control animals. Pigmented animals did not differ in sensitivity or effect compared to albino animals. Mild lesions were described as “retraction and thickening of the outer segments of the photoreceptor cells” while more severe effects included swelling “through the inner segments of the photoreceptor cells to the outer nuclear layer.” Some evidence of inflammatory cell infiltration was also noted and the pigmented epithelial layer was also degenerated. The authors conclude that the effects were attributable to MDA because no retinal lesions have been associated with exposure to the PEG vehicle. Furthermore, the inhalation exposures to MDA are the likely cause rather than the dermal and lung sensitization study exposures because these subsequent studies were conducted on control as well as treated animals. Pulmonary granulomas consisting of “an aggregate of macrophages surrounded by a thin mantle of lymphocytes” were found in 7 of the 16 MDA-exposed animals and one of the 8 control animals (level of significance was not stated). Treated and control animals had a high background incidence of pulmonary lesions including slight to mild bronchitis. No liver or kidney effects were detected in treated animals.

Nine purebred beagle dogs were treated orally (by capsule) with 70 mg “crude” (4 dogs) or “purified” (5 dogs) MDA in corn oil three days per week for a period ranging from approximately 3 to 7 years (Deichmann *et al.*, 1978). No concurrent controls were included since untreated animals were regularly maintained in the laboratory. After 2 years, cystoscopic examination was performed at 15-month intervals. After 4½ years, clinical chemistry tests were performed at 4 month intervals on 3 dogs from each group. Microscopic examination of urinary bladder, liver, heart, ovaries, uterus, and lymph nodes was performed on moribund animals or at the end of the experimental period (7 years, 2 months). Liver toxicity was noted in all the treated animals. Effects were described as fatty change, cell degeneration and necrosis, and lymphoid cell infiltration. One dog from each treatment group died from the toxic effect on the liver. The kidneys of four treated animals (two from each group) showed toxic effects including granuloma, glomerular nephritis, and congestion with cloudy swelling. Two dogs treated with “purified” and one dog treated with “crude” MDA showed toxicity to the spleen described as hemosiderosis and swelling with lymphocyte infiltration.

Wistar rats (5/sex/dose) were treated orally with 0, 0.0083, and 0.083 g MDA/kg body weight in propylene glycol daily for 12 weeks (Pludro *et al.*, 1969). Doses were 1% and 10% of the experimentally determined median lethal dose. No significant changes in body weight or hematological parameters were found, although serum albumin, β -globulin, and γ -globulin were elevated in animals in the 0.083 mg/kg dose group. The livers of all the animals in the high dose group showed signs of degeneration, including atrophy of the parenchyma and stromal hyperplasia in the portal areas. Also in this dose group, all animals showed hypertrophy of the lymphatic nodules of the spleen. In the low dose group, one animal showed a liver lesion and one a lesion in the spleen.

Schoental (1968) treated rats (8/sex) with MDA in 25% aqueous ethanol by stomach tube. Rats were given 20 mg doses a total of 2-5 times over several weeks up to 7½ months (frequency not specified). Animals showed necrosis of the liver and kidney and congestion and edema of the lungs.

Visual toxicity was reported in 15 cats treated perorally with 25-100 mg MDA/kg body weight in a 1% aqueous suspension (Schilling von Canstatt *et al.*, 1966). In four animals treated once with 100 mg/kg, no blindness was reported. In all the other treated animals (four with one dose of 100 mg/kg, two with one dose of 150 mg/kg, and two with three doses of 25 mg/kg and 3 doses of 50 mg/kg), blindness occurred within 8 days. Three of the eight recovered sight within 4 days. Two other treated animals were examined microscopically, one treated with 25 and then 50 mg/kg and one treated once with 200 mg/kg. The first was examined after 7 days and showed signs of granular degeneration of the rods and cones with some proliferation of the pigmented epithelium. The second was examined after 4¼ years and showed atrophy of the retinal neuroepithelium. The authors noted that no visual disturbances were found in other MDA treated experimental animals, including dog, rabbit, guinea pig, and rat.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Leong <i>et al.</i> , 1987
<i>Study population</i>	Guinea pigs
<i>Exposure method</i>	Discontinuous inhalation exposure (nose only) of aerosols
<i>Critical effects</i>	Degeneration of retinal epithelium
<i>LOAEL</i>	440 mg/m ³ (54 ppm)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	4 hours/day, 5 days/week
<i>Exposure duration</i>	2 weeks
<i>Average experimental exposure</i>	52 mg/m ³ for LOAEL group (440 x 4/24 x 5/7) (6.4 ppm)
<i>Human equivalent concentration</i>	52 mg/m ³ using the default assumption of RGDR = 1 for a gas with systemic effects
<i>LOAEL uncertainty factor</i>	10 (incidence = 100%)
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	3,000
<i>Inhalation reference exposure level</i>	0.02 mg/m ³ (20 µg/m ³ ; 0.002 ppm; 2 ppb)

Two specific types of toxicity have been associated with exposure to MDA: hepatotoxicity and ocular toxicity. Several studies have demonstrated hepatotoxicity in experimental animals. The best study of long term toxicity of MDA was the report by Lamb *et al.* (1986). In addition to addressing the carcinogenicity of MDA, Lamb described non-cancer health effects, which resulted from lifetime exposure of two species, rats and mice, to MDA at two concentrations in the drinking water. The 150 ppm dose level was a LOAEL for fatty change and focal cellular

change to the livers of male and female rats as well as for liver degeneration in male mice. The corresponding effects were also observed in high-dose male rats and male mice. Nephropathy was observed in mice of both sexes at the 150 and 300 ppm. There is abundant evidence from both human and animal studies that MDA is hepatotoxic. Bastian (1984), Williams *et al.* (1974), and McGill and Motto (1974) reported hepatitis in people exposed by inhalation and dermal absorption routes. Kopelman *et al.* (1966a,b) demonstrated human hepatotoxicity from exposure by the oral route. However, limited data detailing exposure levels associated with adverse health effects in humans preclude the development of a chronic REL from studies in humans.

The other toxic effect of potential concern from MDA exposure is ocular toxicity. Leong *et al.* (1987) reported damage to the retinas of guinea pigs exposed for 2 weeks to MDA aerosols (0.44 g/m^3 for 4 hr/day, 5 days/week; average experimental exposure = 52 mg/m^3) by inhalation. Schilling von Canstatt *et al.* (1966) also reported blindness in cats treated orally with MDA. A single case of retinopathy and visual toxicity in humans was reported in a man who accidentally ingested MDA with potassium carbonate and γ -butyrolactone. The Leong *et al.* (1986) study was selected for the development of the chronic REL because, although conducted for a relatively short period of time, the study appears to address the most sensitive endpoint of toxicity by the most appropriate route of exposure (inhalation). The studies, which established the hepatotoxicity of MDA, were conducted by the oral route of exposure.

As a comparison with the proposed REL, the study by Lamb *et al.* (1986) found a LOAEL of 9 mg/kg-day for liver changes in male rats. Use of a LOAEL UF of 3, an interspecies UF of 10, and an intraspecies UF of 10 results in an oral chronic REL of 0.03 mg/kg-day. Use of route-to-route extrapolation with the assumption that a 70 kg person breathes 20 m^3 of air per day leads to an inhalation chronic REL estimate of $100 \text{ } \mu\text{g/m}^3$. The proposed chronic REL based on Leong *et al.* (1987) is lower by a factor of 5 than that obtained by using Lamb *et al.* (1986) and should be protective of hepatotoxicity.

VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for 4,4'-methylene dianiline include the availability of a controlled exposure inhalation study. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies, the lack of reproductive and developmental toxicity studies, and the lack of observation of a NOAEL. In addition the test animals were under additional stress due to the restraint used to obtain nose-only exposure, while the control animals were not restrained. Liver toxicity has been included as a potential critical effect because of uncertainty regarding the relative potency of this compound in causing liver toxicity in different species by different routes of exposure.

When assessing the health effects of methylene dianiline, its carcinogenicity must also be assessed.

VIII. Potential for Differential Impacts on Children's Health

No evidence to support a differential effect of methylene dianiline on infants and children was found in the literature.

IX. References

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